67. (Amended) The baculovirus vector of Claim 66, wherein said glycosylphosphatidylinositol anchor coding sequence is a CD59 or a CD14 gene.

REMARKS

Claims 46-53, 55-63 and 65-68 are active in the present application. The claims are amended for clarity and consistent with the Examiner's helpful suggestions set forth in the Office Action. No new matter is believed to be added by these amendments. Favorable reconsideration is respectfully requested.

At the outset, Applicants wish to thank Examiner Grun for indicating that Claim 68 has been found to be allowable. Favorable reconsideration and allowance of all claims is requested.

The issues raised in the Official Action (Paper No. 23) are addressed in turn below.

1. The rejection of Claims 46-50, 53, 56-63 and 65-67 under 35 U.S.C. § 112, first paragraph is respectfully traversed.

As a basis of this rejection, the Examiner asserts that only the *Plasmodium* MSP-1 signal sequence is taught by the instant Applicants and that "the ability of another signal sequence to result in appropriate expression of the protein would seem to be unknown and unpredictable" (Page 3 of the Official Action). Applicants respectfully disagree but note that the instant claims as amended herewith define the signal sequence as that from *Plasmodium* MSP-1.

Accordingly, withdrawal of this ground of rejection is requested.

2. The rejection of Claim 56, 58 and 61 under 35 U.S.C. § 102(b) over Longacre et al (Mol. Biochem. Parasitol. 64:191 (1994)) is respectfully traversed.

The claims have been amended to recite "*Plasmodium falciparum* MSP-1 protein" and submit that such synthetic polynucleotide encoding said protein is not anticipated by Longacre et al because Longacre et al merely discloses a recombinant baculovirus containing a DNA fragment of *Plasmodium vivax* MSP-1. However, the *Plasmodium falciparum* MSP-1 in the instant claims and the Longacre et al *Plasmodium vivax* MSP-1 are not the same (see, Applicants' Amendment and Request for Reconsideration filed August 3, 2000 and the Dr. Longacre-Andre Declaration)

Accordingly, withdrawal of this ground of rejection is respectfully requested.

3. The rejection of Claims 46, 48, 55, 56, 58, 61 and 65-66 under 35 U.S.C. § 103(a) over Chappel et al, Miller et al and Longacre et al is respectfully traversed.

As a basis of this rejection the Examiner has alleged that the skilled artisan would have cloned and expressed the sequence encoding the C-terminal fragment of MSP-1 protein of any *Plasmodium* species. Applicants respectfully disagree and note that the instant claims as amended herein recite "a 19 kilodalton C-terminal fragment of a *Plasmodium falciparum* merozoite surface protein 1 . . ." In addition, Applicants point out that the instant claims are drawn to an altered sequence of the MSP-1 protein whereby the GC content of the native sequence has been increased to 40% to 60%, which is different from the native *P. falciparum* sequence of 33% as noted in the Longacre Declaration already of record. The specification further discloses that 57 of the 93 codons of the native *P. falciparum* sequence were modified (page 16 of the instant specification). This synthetic polynucleotide and protein is not

disclosed or suggested in the cited references because the cited references simply fail to suggest these modifications.

Chappel et al disclose the direct expression of individual EGF-like domains from the C-terminus of the MSP-1 protein as a fusion protein in *Escherichia coli*. The plasmid employed was pGEX-3X (see page 304, col. 1 of Chappel). As evidenced in the attached description of this vector, this vector contains the glutathione S-transferase gene region and an internal I^q gene to facilitate *E. coli* expression. In addition, Chappel et al teach that the some of expressed EGF-like domains failed to interact with the tested antibodies, which is indicative that a tertiary or other higher order structure may be required that is not attained when expressed in *E. coli* (referring to the discussion o page 308, column 2 of Chappel).

Chappel et al simply fail to disclose any modification of a 19 kilodalton C-terminal fragment of *Plasmodium falciparum* MSP-1 to yield a protein having a GC content from 40 to 60%. Likewise, Chappel et al are silent with respect to utilizing a signal sequence in a baculovirus vector as claimed.

Miller et al teach the alignment and sequence diversity of previously known MSP-1 parasite clones. However, Miller et al fail to teach modifying a 19 kilodalton C-terminal fragment of *Plasmodium falciparum* MSP-1 to yield a protein having a GC content from 40 to 60%, a baculovirus vector or a *Plasmodium* MSP-1 signal sequence as claimed.

Longacre et al teach the expression of *Plasmodium vivax* in baculovirus, but as noted with respect to the rejection under 35 U.S.C. § 102, Longacre et al fail to teach modifying a 19 kilodalton C-terminal fragment of *Plasmodium falciparum* MSP-1 to yield a protein having a GC content from 40 to 60%.

Therefore, in light of the clear fact that the three cited references fail to disclose or suggest the *P. falciparum*, MSP-1 protein having a GC of 40 to 60%, the instant claims

cannot be obvious in view of Chappel et al, Miller et al and Longacre et al. In addition, Applicants have already made of record clear evidence that The the protein encoded by the synthetic gene of a *Plasmodium falciparum* having a GC content of 40 to 60% exhibited significantly more reactivity with hyperimmune antiserum than the native gene which did not meet this criteria.

Accordingly, withdrawal of this ground of rejection is respectfully requested.

4. The rejection of Claims 46-53, 55-63 and 65-67 under 35 U.S.C. § 112, second paragraph is obviated by amendment.

The claims have been amended as suggested by Examiner Grun. Accordingly, withdrawal of this ground of rejection is requested.

Applicants submit that the present application now stands in condition for allowance.

Early notification of such allowance is earnestly solicited.

Respectfully submitted,
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IN THE CLAIMS

Please amend the claims as follows:

46. (Twice Amended) A baculovirus vector comprising a promoter, a synthetic polynucleotide encoding a 19 kilodalton C-terminal fragment of a *Plasmodium falciparum* [MSP-1 protein; and] merozoite surface protein 1 (MSP-1), wherein said synthetic polynucleotide has a GC content of 40 % to 60% and a polynucleotide encoding a signal peptide [wherein said signal peptide is from an] of a *Plasmodium* MSP-1 protein.

48. (Amended) The baculovirus vector of Claim 46, wherein said synthetic polynucleotide further comprises a glycosylphosphatidylinositol anchor coding sequence.

49. (Twice Amended) The baculovirus vector of Claim 48, wherein said glycosylphosphatidylinositol anchor coding sequence is from a CD59 or CD14 gene.

55. (Twice Amended) A baculovirus vector selected from the group consisting of PfMSP1p19A deposited at the CNCM under No. I-1661, PfMSP1p19S deposited at the CNCM under No. I-1662, and PcMSP1p19S deposited at the CNCM under No. 1663.

56. (Twice Amended) A synthetic polynucleotide comprising a gene encoding a 19 kilodalton C-terminal fragment of a *Plasmodium* [MSP-1] *falciparum* merozoite surface protein (MSP-1) polypeptide[;] , wherein said polynucleotide has a total GC content of 40 % to 60%.

58. (Amended) The synthetic polynucleotide of Claim 56, wherein said synthetic polynucleotide further comprises a glycosylphosphatidylinositol anchor coding sequence.

59. (Twice Amended) The synthetic polynucleotide of Claim [56] 58, wherein said glycosylphosphatidylinositol anchor coding sequence is from a CD59 or CD14 gene.

61. (Amended) The synthetic polypeptide of Claim 56, wherein said synthetic polynucleotide further comprises a polynucleotide encoding a signal peptide of a *Plasmodium* MSP-1 protein.

65. (Amended) A baculovirus vector comprising a promoter, a synthetic polynucleotide encoding a 19 kildalton C-terminal fragment of a *Plasmodium falciparum* [MSP-1 protein] merozoite surface protein 1 (MSP-1) having a GC content of between 40% to 60% and a polynucleotide encoding a signal sequence [from] of a *Plasmodium vivax* MSP-1 protein.

66. (Amended) The baculovirus vector of Claim 65, wherein said synthetic polynucleotide sequence further comprises a glycosylphosphatidylinositol anchor coding sequence.

67. (Amended) The baculovirus vector of Claim 66, wherein said glycosylphosphatidylinositol anchor coding sequence is a CD59 or a CD14 gene.

Annex I

pGEX Vectors (GST Gene fusion)

Ordering information

Product	Quantity	Code Number	Price £
Glutathione S-transferase Gene Fusion Vectors*			
pGEX-1λT *Eco*R I/BAP	5 µg	27-4805-01	POR
pGEX-2T	25 µg	27-4801-01	POR
pGEX-2TK	25 µg	27-4587-01	POR
pGEX-3X	25 µg	27-4803-01	POR
pGEX-4T-1	25 µg	27-4580-01	POR
pGEX-4T-2	25 µg	27-4581-01	POR
pGEX-4T-3	25 µg	27-4583-01	POR
pGEX-5X-1	25 µg	27-4584-01	POR
pGEX-5X-2	25 µg	27-4585-01	POR
pGEX-5X-3	25 µg	27-4586-01	POR
pGEX-6P-1	25 µg	27-4597-01	POR
pGEX-6P-2	25 µg	27-4598-01	POR
pGEX-6P-3	25 µg	27-4599-01	POR

** All vectors include E. coli BL21 cells.*

All of the GST gene fusion vectors offer:

- ***A tac promoter for chemically inducible, high-level expression.***
- An internal *lac* I^q gene for use in any *E. coli* host.
- Very mild elution conditions for release of fusion proteins from the affinity matrix, thus minimizing effects on antigenicity and functional activity.
- PreScission, thrombin, or factor Xa protease recognition sites for cleaving the desired protein from the fusion product.

Thirteen pGEX vectors are available (see figure). Nine of the vectors have an expanded multiple cloning site (MCS) that contains six restriction sites. The expanded MCS facilitates the unidirectional cloning of cDNA inserts obtained from libraries constructed using many available lambda vectors including λ ExCell Cloning Vector (27-5013-01 or 27-5011-01;see λ ExCell *Not* I/*Eco*R I/CIP
λ ExCell *Eco*R I/CIP for more details) and Lambda ZAP. pGEX-6P-1, pGEX-6P-2 and pGEX-6P-3 each encode the recognition sequence for site-specific cleavage by PreScission Protease (27-0843-01; see PreScission Protease) between the GST domain and the multiple cloning site. pGEX -4T-1, pGEX-4T-2 and pGEX-4T-3 are derived from pGEX-2T and contain a thrombin recognition site. pGEX-5X-1, pGEX-5X-2, and pGEX5X-3 are derivatives of pGEX-3X and possess a factor Xa recognition site.

pGEX-2TK is uniquely designed to allow the detection of expressed proteins by directly labelling the fusion products *in vitro* (1). This vector contains the recognition sequence for the catalytic subunit of cAMP-dependent protein kinase obtained from heart muscle. The protein kinase site is located between the GST domain and the MCS. Expressed proteins can be directly labelled using protein kinase and [γ-^{32}P]ATP and readily detected using standard radiometric or autoradiographic techniques. pGEX-2TK is a derivative of pGEX-2T; its fusion proteins can be cleaved with thrombin.